THE RESISTANCE OF *STREPTOCOCCUS PNEUMONIAE* AGAINST PENICILLIN AND OTHER ANTIBIOTICS

S.A.H. Jahanmehr[1], A. Rajabi[*2], M. Soltani Radd[2] and Gh. Behzadian Nejad[3]

1) Paramedical Faculty, Tehran University of Medical Sciences, Tehran, Iran
2) Central Laboratory, Imam Khomeini Medical Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3) Medical Faculty, Tarbiat Modarress University of Medical Sciences, Tehran, Iran

Abstract- Resistance of *Streptococcus pneumoniae* against penicillin is considered to be of great importance. While low-resistant strains could be treated by penicillin, treatment of highly resistant strains is very difficult and needs broad-spectrum antibiotics. This study was performed in Imam Khomeini Medical Center, Tehran, Iran, from 1999 to 2001 to evaluate pneumococcal resistance against penicillin and some other antibiotics. Specimens were collected from different hospitals. Samples were cultured and resistance of *S. pneumoniae* against selected antibiotics was determined. The main aim of this study was to measure minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), using serial dilution method. Disc diffusion method was also performed to be compared with the main procedures (MIC and MBC), on 66 strains obtained from 100 clinical specimens. Five different antibiotics (penicillin, cefazolin, ampicillin, amoxicillin and vancomycin) were employed in this study. In the case of penicillin, 47 sensitive strains and 19 highly resistant strains were obtained. No intermediate or low-resistant strain was found. The frequency of resistant strains against other antibiotics was found to be 10.6%, 7.5%, 18.1% and 0%, respectively. All strains were sensitive to vancomycin except for a low resistant one. Care should be taken to choose a suitable drug when being faced with a resistant strain. Vancomycin can be used with confidence to cure infections induced by penicilin-resistant *S. pneumoniae*.

Acta Medica Iranica, 42(3): 223-227; 2004

Key words: Resistant *Streptococcus pneumoniae*, MIC, MBC

INTRODUCTION

Infectious diseases such as meningitis, otitis media, sinusitis and septic arthritis are mostly caused by *Streptococcus pneumoniae*. In United States, there are 3000 cases of meningitis, 50,000 bacteremia, 500,000 pneumonia and seven millions cases of otitis media per year caused by *S. pneumoniae* (1). The invasive diseases caused by these bacteria are found to be very important, particularly in the areas where prevalence of *Hemophylus influenza* type b is markedly reduced by immunization (2).For many years, penicillin was used as the treatment of choice for pneumococcal infections. But in 1967 the first penicillin resistant strain was seen in Australia (3) and frequency of resistant strains has risen steadily since then. In Canada, penicillin-resistance of this microorganism increased from 1977 to 1990, so that it reached up to 1.5% in Ontario, 2.4% in Alberta and 1.3% in Quebec (4). Another study in Belgium revealed that the resistance of these bacteria to penicillin from 2.3% in 1993 increased to 7.6% in 1994 (5). One study in Brazillia showed that the pneumococcal resistance to penicillin increased from

Received: 24 Dec. 2001, Revised: 4 Jan. 2003, Accepted: 25 Jun. 2003

*** Corresponding Author:**
A. Rajabi, Central Laboratory, Imam Khomeini Medical Center, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 6956919, 6401294
Fax: +98 21 6434020
E-mail: rajabisa@sina.tums.ac.ir

9.6% in 1993 to 20.6%, in 1996 (6).

The problem of bacterial resistance is more prominent among children and it is therefore recommended that health care centers arrange for further vaccination of the children against *S. pneumoniae* (7). Multiple drug resistance of these bacteria is another important problem (8-12). In a study by Thornsberry *et al. S. pneumoniae* showed a significant resistance against all but 3 from a total of 26 antibiotics under investigation (13). Other studies, based on results of microbiological examinations, show that the frequencies of penicillin-resistant strains are increasing in different parts of the world (14-19). The performance of the susceptibility test is therefore recommended (20).

In our previous study, we found 40 pneumococcal resistant strains against penicillin in patients referring to Central Laboratory, Imam Khmeini Medical centre, in Tehran (21). In present study the resistance, as well as the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of selected antibiotics, were eva1uated on strains of *S. pneumoniae.*

MATERIAL S AND METHODS

This work was carried out in Central Laboratory Imam Khomeini Medical Center, Tehran, Iran. Samples obtained from more than one hundred patients referred to different hospitals in Tehran from 1999 to 2001. These samples were cultured and the bacteria were determined using different criteria such as morphology, presence of capsules, hemolysis, susceptibility to optochin and bile solubility (5,6).

There are different criteria by which high resistance, intermediate resistance and sensitive states can be evaluated. Generally, strain is considered highly resistant to penicillin when the MIC is equal or more than 2.0 microgram/ml, intermediately resistant when MIC is 0.12 to 1 microgram/ml and sensitive when MIC is less than 0.12 microgram/ml (22). MIC and MBC were determined by serial dilution method, using modified Muller Hinton broth (Antec Co. U.K), which contains the ions necessary for bacterial growth. We selected penicillin, ampicillin, cefazolin, amoxicillin and vancomycin (Bristol Co. U.K.) in this study.

Suspensions of bacteria, equal to 0.5 McFarland standard tube were made and one ml of their 1/100 dilution was added to each tube, containing the serial diluted antibiotic. After a period of 24 hour incubation, the tubes were observed for any turbidity and the first clear tube was accepted as MIC.

Bacterial samples of all tubes containing the clear solutions were cultured on chocolate agar and the test for colony count was performed. The number of colonies was compared with the colony count of the appropriate tube with original dilution. The minimum concentration of antibiotic that let the growth of less than 0.1 % of bacteria was accepted as MBC (3,4).

The comparison of MIC results with antibiotic susceptibility by disc diffusion method was carried out, using antibiotic discs (Padtan Teb, Tehran, Iran). Bacterial suspensions with the turbidity equal to 0.5 McFarland (tube no. 1) were made and used for the test. Chocolate agar media, cultured with bacterial suspensions were incubated at 37°C for 24 hours. The diameter of inhibited zones was then measured.

RESULTS

During the present study 66 strains of *S. pneumoniae* were isolated from 100 different clinical specimens (Table 1). The results of bacterial resistance against 5 different selected antibiotics, evaluated by disc diffusion technique and serial dilution method, are shown in tables 2 and 3.

Based on disc diffusion technique, 75.7% of strains showed sensitivity to penicillin, whereas frequency of sensitive strains by serial dilution method was 71.2% (MIC<0.12 mg/ml).

Table 1. Absolute and relative frequency of isolated strains

Samples	Number	Percentage
Blood	36	54.5
CSF	12	18.2
Sputum	7	10.6
Body fluids	6	9.09
Eye	4	6.06
Foot wound	1	1.5
Total	66	100

Table 2. Absolute and relative frequency of sensitivity of strains by disc diffusion technique

	Sensitive		Intermediate		Resistant	
Antibiotic	**Number**	**%**	**Number**	**%**	**Number**	**%**
Penicillin	50	75.7	4	6.06	12	18.1
Cefazolin	53	80.3	6	9.09	7	10.6
Ampicillin	55	83.3	6	9.09	5	7.5
Amoxicilin	48	72.7	6	9.09	12	18.1
Vancomycn	65	98.4	1	1.5	0	0

Table 3. Absolute and relative frequency of sensitivity of strains by serial dilution method, as obtained from the results of MIC*

	Sensitive		Intermediate		Resistant	
Antibiotic	**Number**	**%**	**Number**	**%**	**Number**	**%**
Penicillin	47	71	0	0	19	28.78
Cefazolin	44	66.6	7	10.6	15	22.7
Ampicillin	55	83.3	6	9.09	5	7.5
Amoxicillin	48	72.7	6	9.09	12	18.1
Vancomycin	65	98.4	1	1.5	0	0

Abbreviation: MIC, minimum inhibitory concentration.

* Results of MIC and MBC were almost identical, except for cefazolin.

Table 4. Absolute and relative frequency of sensitivity of penicillin-resistant strains by serial dilution method, as obtained from the results of MIC*

	Sensitive		Intermediate		Resistant	
Antibiotic	**Number**	**%**	**Number**	**%**	**Number**	**%**
Cefazolin	0	0	2	10.5	17	89.4
Ampicillin	3	15.7	2	10.5	14	73.7
Amoxicillin	1	5.2	1	5.2	17	89.4
Vancomycin	18	94.7	1	5.2	0	0

Abbreviation: MIC, minimum inhibitory concentration.

* Results of MIC and MBC were almost identical, except for cefazolin.

More than 98% of strains were sensitive to vancomycin in both serial dilution and disc diffusion techniques. Resistance of penicillin resistant strains against other antibiotics was also evaluated. The results of this evaluation are shown in table 4.

DISCUSSION

Penicillin has been considered to be the treatment of choice for infections caused by *S. pneumoniae* for many years. The first resistant strain was reported in 1967 and since then frequency of resistant strains of this bacteria is increasing each year (3-6,14-19), not only against penicillin, but also against many other antibiotics (13).

In Iran, evaluation of penicillin resistance of this bacteria has not gained much favor. This work is therefore carried out to assess the rate of pneumococcal resistance in various infections. In the present study comparison of the disc diffusion technique with the serial dilution method revealed

that results of disc diffusion method and MIC are more compatible in case of penicillin and vancomycin, as compared to ampicillin, amoxicillin and cefazolin. Because of being more practical, rapid and easier, disc diffusion technique is more suitable for routine laboratory works in evaluating the sensitivity of *S. pneumoniae*. In this study results indicated that among antibiotics which are effective against *S. pneumoniae*, cefazolin has the lowest MIC. Vancomycin appears to be a suitable substitution for penicillin. The results obtained from vancomycin were interesting. Antibiotic discs of vancomycin showed that there was only one intermediate resistant strain among 66 studied *S. pneumoniae* strains, and about 98% of strains were susceptible to vancomycin. The same result was also shown in another study (23). Schwartz and Tunkle used vancomycin with or without rifampicin as a therapeutic protocol against meningitis caused by penicillin-resistant *S. pneumoniae* (24).

Bacterial resistance pattern in this study shows that nowadays the resistant strains are more frequently isolated from clinical samples. Hence care should be taken to choose a more suitable drug when there is a possibility of being faced with a resistant strain. We conclude that vancomycin can be used with confidence to cure an infection induced by penicilin-resistant *S. pneumoniae*.

Acknowledgments

We would like to appreciate the technical assistance given, during this study, by Mahfoozi S, Rajabi E, and Zolfaghari A. Central Laboratory, Imam Khomeini Medical Centre, Tehran, Iran.

REFERENCES

1. No authors listed. Emergence of penicillin-resistant Streptococcus pneumoniae--southern Ontario, Canada 1993-1994. MMWR Morb Mortal Wkly Rep. 1995; 44(11): 207-209.

2. Giebink, GS, Foker, J.E, Kim, Y, Schiffman, G. Serum antibody and opsonic responses to vaccination with pneumococcal capsular polysaccharide in normal and splenectomized children. J Infect Dis. 1980; 141(3): 404-412.

3. Dagan R, Yagupsky P, Goldbart A, Wasas A, Klugman K. Increasing prevalence of penicillin-resistant pneumococcal infections in children in southern Israel: implications for future immunization policies. Pediatr Infect Dis J. 1994; 13(9):782-786.

4. Kaplan SL. The emergence of resistant pneumococcus as a pathogen in childhood upper respiratory tract infections. Semin Respir Infect. 1995; 10 (1): 31-36.

5. Allen KD. Penicillin- resistant Pneumococci. J Hosp Infect. 1991; 17(1): 3-13.

6. Hoefnagels-Schuermans A, Van Eldere J, Van Lierde S, Verbist L, Verhaegen J, Peetermans WE. Increase in penicillin resistance rates in Belgium due to clonal spread of a penicillin-resistant 23F streptococcus pneumoniae strain. Eur J Clin Microbiol Infect Dis. 1999; 18(2):120-125.

7. Brandileone MC, Di Fabio JL, Vieira VS, Zanella RC, Casagrande ST, Pignatari AC, Tomasz A. Geographic distribution of penicillin resistance of Streptococcus pneumoniae in Brazil: genetic relatedness. Microb Drug Resist. 1998; 4(3): 209-217.

8. Fotopoulou N, Tsiplakou S, Maniatis AN, Pangali A, Kouppari G, Legakis NJ, Tassios PT. Gradual increase in the minimum inhibitory concentration of penicillin among both susceptible and resistant Streptococcus pneumoniae isolated from Greek children during 1995 – 1997. Int J Antimicrob Agents. 1999; 11(1): 53-57.

9. Geslin P, Fremaux A, Sissia G, Spicq C, Georges S. Development of resistance to beta-lactams and other antibiotics of pneumococci isolated from acute otitis media in France: Statement of the National Reference Center, 1995-1996. Arch Pediatr. 1998; 5(9):982-987.

10. Dombert MC, Aubier M. Pneumococci with diminished sensitivity to penicillin in pneumopathies: clinical consequences. Presse Med. 1998; 27(12): 583-587.

11. Reichler MR, Rakovsky J, Slacikova M, Hlavacova B, Krajcikova L, Tarina P, Sobotova A, Facklam RR, Breiman RF. Spread of multidrug-resistant Streptococcus pneumoniae among hospitalized children in Slovakia. J Infect Dis. 1996; 173(2): 374-379.

12. Nissinen A, Leinonen M, Huovinen P, Herva E, Katila ML, Kontiainen S, Liimatainen O, Oinonen S, Takala AK, Makela PH. Antimicrobial resistance of Streptococcus pneumoniae in Finland, 1987-1990. Clin Infect Dis. 1995; 20(5):1275-80.

13. Kam KM, Luey KY, Fung SM, Yiu PP, Harden TJ, Cheung MM. Emergence of multiple-antibiotic-resistant Streptococcus pneumoniae in Hong Kong. Antimicrob Agents Chemother. 1995; 39(12): 2667-2670.

14. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 11th ed. St. Louis: Mosby Company; 2002. p. 229-250.

15. Koornhof HJ, Wasas A, Klugman K. Antimicrobial resistance in Streptococcus pneumoniae: A South African prespective. Clin Infect Dis. 1992; 15(1): 84-94.

16. Marton A. Pneumococcal antimicrobial resistance: the problem in Hungary. Clin Infect Dis. 1992; 15(1): 106-111.

17. Linares J, Pallares R, Alonso T, Perez JL, Ayats J, Gudiol F, Viladrich PF, Martin R. Trends in antimicrobial resistance of clinical isolates of Streptococcus pneumoniae in Bellvitge Hospital, Barcelona, Spain. Clin Infect Dis. 1992; 15(1): 99-105.

18. George RC, Ball LC, Cooper PG. Antibiotic-resistant pneumococci in the United Kingdom. Commun Dis Rep CDR Rev. 1992; 2(4): R37-43.

19. Block SL, Harrison CJ, Hedrick JA, Tyler RD, Smith RA, Keegan E, Chartrand SA. Penicillin-resistant Streptococcus pneumoniae in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. Pediatr Infect Dis J. 1995; 14(9): 751-759.

20. Thornsberry C, Ogilvie PT, Holley HP Jr, Sahm DF. In vitro activity of grepafloxacin and 25 other antimicrobial agents against Streptococcus pneumoniae: correlation with penicillin resistance. Clin Ther. 1998; 20(6): 1179-1190.

21. Rajabi A, Jahanmehr SAH, Soltani Rad M, Behzadian Nejad Gh. Evaluation of pneumoccocal resistance to penicillin, in patients referring to Central Laboratory, Imam Khomeini Medical Centre. Tashkhis Azmayeshgahi (Laboratory Diagnosis). 2001; 15: 42–46.

22. Boken DJ, Chartrand SA, Goering RV, Kruger R, Harrison CJ. Colonozation with penicillin-resistant Streptococcus pneumoniae in a child-care center. Pediatr Infect Dis J. 1995; 14(10): 879-884.

23. Bergan T, Gaustad P, Hoiby EA, Berdal BP, Furuberg G, Baann J, Tonjum T. Antibiotic resistance of pneumococci in Norway. Int J Antimicrob Agents. 1998; 10(1): 77-81.

24. Schwartz MT, Tunkel AR. Therapy of penicillin-resistant pneumococcal meningitis. Zentralbl Bakteriol. 1995; 282 (1): 7-12.